Dietary Vitamin D3, the Skin Immune System and implications for Skin Cancer

by

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Declaration of Originality

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Abstract

Ultraviolet radiation (UVR) causes mutagenic DNA damage to skin cells and suppression of the skin immune system (SIS) that contributes to skin cancer development. UVR in the UVB range also causes the beneficial production of vitamin D3 by keratinocytes. Keratinocytes also contain the enzymes required for the two-step hydroxylation of vitamin D3 is to its biologically active form: 1α,25 dihydroxyvitamin D3 (1α,25 $(OH)_2$D3). 1α,25 $(OH)_2$D3 has been demonstrated to modulate the SIS and its response to UVB irradiation. Topical application of 1α,25 $(OH)_2$D3 suppresses the SIS and protects against UV-induced DNA damage. This thesis investigates the hypothesis that dietary vitamin D3 similarly suppresses the SIS response via modulation of the SIS and protects against UVB induced immunosuppression via a reduction in UVB induced DNA damage.

To assess the influence of vitamin D3 on the development and function of the SIS, and the skins response to UVR, a vitamin D3 deficient mouse population was developed. Breeding pairs of vitamin D3 replete and vitamin D3 deficient mice were formed and the off-spring of these mice were used in experiments. The contact hypersensitivity (CHS) response was used to assess the SIS function in vitamin D3 replete and vitamin D3 deficient mice. In this model the application of the contact sensitiser to the skin of neonatal mice leads to suppression of the CHS response upon subsequent re-sensitisation and elicitation of the CHS in adulthood. The suppression of the CHS response is termed ‘neonatal tolerance’.

Vitamin D3 enhanced neonatal tolerance in male but not female mice. The enhanced neonatal tolerance induction related to an increase in the proportion of $CD4^+CD25^+$ cells in vitamin D3 replete neonatal male mice and this cell population could transfer tolerance in this mouse group. Vitamin D3 suppressed the CHS response in adult male but not adult female mice. An investigation of DC function in adult mice revealed no modulation by vitamin D3 in antigen carriage or co-stimulatory molecule expression, suggesting that these cells were not modulating the reduced CHS response in adult male mice. However, dietary vitamin D3 was associated with a higher proportion of

$CD4^+CD25^+$ cells in the SDLN after contact sensitiser application in male but not female mice, implying that vitamin D3 was modulating the induction of these cells.

The level of UVB-induced immunosuppression was assessed in vitamin D3 replete and deficient mice in C57BL/6 and BALB/c mice. Vitamin D3 protected C57BL/6, but not BALB/c mice against UVB-induced immunosuppression. The protection against UVB-induced immunosuppression may relate to a reduction in UVB-induced DNA damage in C57BL/6 mice, not identified in BALB/c mice. In BALB/c mice female gender protected against UVB-induced immunosuppression and was associated with a subtle lowering of UVB-induced DNA damage in comparison to male mice at low UVR exposures. These results indicate that the protection against UVB-induced immunosuppression by vitamin D3 varies with genetic background and the modulation of UVB-induced DNA damage.

If these mouse studies are extended to humans, the ability of vitamin D3 to suppress the SIS and the limited protection against UVB-induced DNA damage in males, depending on their genetic background, may contribute to the greater burden of skin cancer in males than in females.

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Commonly Used Abbreviations

APC	Antigen Presenting Cells
BCC	Basal Cell Carcinoma
CHS	Contact Hypersensitivity
ConA	Concanavalin A
CCR	Chemokine Receptor
CCL	Chemokine Ligand
cDC	conventional or classical Dendritic Cell
CTLA-4	Cytotoxic T-lymphocyte Antigen 4
CLA	Cutaneous Lymphocyte Antigen
CPD	Cyclobutane Pyrimidine Dimer
DC	Dendritic Cells
7-DHC	7- dehydrocholesterol
EAE	Experimental Autoimmune Encephalomyelitis
FITC	Fluoroscein Isothiocyanate
Flt3	fms-related tyrosine kinase 3
IBD	Inflammatory Bowel Disease
IFN	Interferon
IL	Interleukin
iNKT	invariant Natural Killer T cell
iTreg	induced regulatory T cell
LC	Langerhans cell
MCP-1	Monocyte Chemoattractant Protein- 1
MHC	Major Histocompatibility Complex
MMP	Matrix Metalloproteinase
MS	Multiple Sclerosis
MT	Metallothionein
NK	Natural Killer
NMSC	Non-Melanoma Skin Cancer
NOD	Non-obese Diabetic
nTregs:	naturally occurring regulatory T cells

PAF	Platelet Activating Factor
pDC	plasmacytoid Dendritic Cell
PGE2	Prostaglandin E2
RANK	Receptor Activator for Nuclear Factor κ B
RANKL	Receptor Activator for Nuclear Factor κ B Ligand
ROS	Reactive Oxygen Species
SDLN	Skin Draining Lymph Nodes
SEM	Standard Error of the Mean
SD	Standard Deviation
SCC	Squamous Cell Carcinoma
SIS	Skin Immune System
TD	Thymine Dimers
TCR	T Cell Receptor
Th	T helper
TGF-β	Transforming Growth Factor β
Treg	Regulatory T cell
UCA	Urocanic Acid
UV	Ultraviolet
UVR	Ultraviolet Radiation
VDR	Vitamin D Receptor

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Foreword

Skin cancer is the most common cancer in the world. The majority of skin cancers are caused by exposure of the skin to ultraviolet B radiation (UVB). UVB irradiation causes DNA damage to cells of the skin and suppression of the skin immune system (SIS). UVB irradiation also causes the production of vitamin D3 by skin keratinocytes. Vitamin D3 is enzymatically hydroxylated to produce 25(OH)D3, which is the major circulating form of vitamin D3. 25(OH)D3 is then available for a further hydroxylation to become it's biologically active form 1α,25 dihydroxyvitamin D3 (1α,25$(OH)_2$D3) by a variety of cells including cells of the skin and the SIS. Keratinocytes of the skin produce 1α,25$(OH)_2$D3 after UVB irradiation and this has led to the discovery that 1α,25$(OH)_2$D3 can suppress the function of key cells of the SIS and protects skin against UVB-induced DNA damage and UVB-induced immunosuppression. This may contribute to a reduced risk of skin cancer development. The focus of this study was to determine whether dietary vitamin D3, and therefore circulating levels of 25(OH)D3 available to be converted to 1α,25$(OH)_2$D3, similarly influenced the development and function of the SIS and the skins response to UVB-irradiation.

A vitamin D3 deficient mouse population was developed to elucidate the role of dietary vitamin D3 on the development and function of the SIS and response to UVB-irradiation. An immune response known as the contact hypersensitivity (CHS) response, generated by the SIS upon skin exposure to an antigen (contact sensitiser) was compared in vitamin D3 replete and vitamin D3 deficient mice. The investigation of the development and function of the SIS involved an assessment of the directors of the CHS response, dendritic cells (DC); the effectors of the CHS response, T and B cells; and regulators of the CHS, regulatory T cells. UVB-induced suppression of the SIS was also investigated to ascertain the role of dietary vitamin D3 on the skins response to UVB-irradiation. Vitamin D3 subtly altered DC, CHS effectors and regulatory T cells in an age and gender specific manner. Similarly vitamin D3 protected against UVB-induced immunosuppression in a gender and mouse strain dependent manner. As vitamin D3 deficiency and skin cancer are common, it is important to clarify how vitamin D3 modulates cutaneous biology and how this may relate to skin cancer development.